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(54) Title: CYCLIC HEXAPEPTIDES AS TACHYQUININ ANTAGONISTS, THEIR PREPARATION AND PHARMACEUTICAL COMPOSITIONS THEREOF

$$\boxed{\text{NH-CHR}_1\text{-W-CHR}_3\text{-CO-A}_1\text{-A}_2\text{-A}_3\text{-}\beta\text{Ala}} \qquad \text{(I)}$$

(57) Abstract

A description is given of hexapeptide analogues of tachyquinines and their pharmaceutically acceptable salts as per general formula (I) effective in the treatment of diseases, where tachyquinines play a pathogenetic role, in particular in the treatment of arthritis, asthma, inflammations, tumoral growth, gastrointestinal hypermotility, Huntington's disease, neuritis, neuralgia, migraine, hypertension, incontinence of urine, urticaria, carcinoid syndrome symptoms, influenza, and cold.

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CYCLIC HEXAPEPTIDES AS TACHYKININ ANTAGONISTS, THEIR PREPARATION AND PHARMACEUTICAL COMPOSITIONS THEREOF

Field of the invention

The invention refers to cyclic hexapeptide analogues of tachykinin s of general formula (I)

(I) $\boxed{NH\text{-}CHR_1\text{-}W\text{-}CHR_3\text{-}CO\text{-}A_1\text{-}A_2\text{-}A_3\text{-}\beta Ala}$

where

R_1 = H, linear or branched C_{1-4} alkyl

R_3 = H, natural or not natural amino acid free or protected sid chain

or

R_3 = $(CH_2)_n$-R"

wherein

n = 1, 2, 3, 4, 5

R" = cyclooctyl, adamantyl, cyclohexyl, naphthyl

R" = phenyl when n is other than 1

R" = a substituted carboxyamide group when n = 1, 2

A_1 = Gln, DGln

A_2 = Trp, DTrp

A_3 = Ph , DPhe

W = CO-NR', CH_2-NR'

where

R' = H, CH_3 and their pharmaceutically acceptable salts with acids.

or organic or inorganic bas s.

Tachykinins antagonist compounds of formula (I) prove to b effective in the treatment of diseases where tachykinins play a pathogenic role, in particular in the treatment of arthritis, asthma, inflammations, tumor growth, gastrointestinal hypermotility, Huntington's disease, neuritis, neuralgia, migraine, hypertension, incontinence of urine, urticaria, carcinoid syndrome symptoms, influenza, and cold.

State of the Art

Tachykinins are a family of peptides characterized by the following common C-terminal sequence:

Phe-X-Gly-Leu-Met-NH_2

where X stands for an amino acid characterizing each of th tachykinins.

As far as mammals are concerned, the three tachykinins were called substance P (SP) (where X = Phe), neurokinin A (NKA) (where X = Val) and neurokinin B (NKB) (where X = Val) and their neurotransmitter role, both at peripheral and central levels, was acknowledged (J.E. Maggio, Peptides, 1985, 6, 237-245 and P.C. Emson et al., Neuropeptides and their peptidases, A.J. Turner and Ellis Horwood, England, 1987, pp. 87-106).

The pharmacological and biochemical results conveyed by the literature show that the biological activity of tachykinins is mediated, in mammals' tissues, by three distinct receptors at least, called NK-1, NK-2, NK-3. Natural tachykinines exhibit a different

affinity with such three r c ptors. Highly p t nt tachykinins antagonists seem to be effective to reduce or antagoniz pathological effects due to an excess of tachykinins in animals or man. The first generation tachykinins antagonists described, for instance, in US-A-4,481,139 - scarcely selective - were followed by the second generation ones (EP-A-401,177; EP-A-347,802; GB-A-2,216,529), more selective.

Research in the field is anyway aimed at singling out high r affinity and activity antagonists, free from agonist activity n other receptors, hence suitable for therapeutical use.

Detailed Description of the Invention

This invention refers to cyclic hexapeptide analogues of tachykinins of general formula (I)

(I) $\boxed{\text{NH-CHR}_1\text{-W-CHR}_3\text{-CO-A}_1\text{-A}_2\text{-A}_3\text{-}\beta\text{Ala}}$

where

R_1 = H, linear or branched C_{1-4} alkyl

R_3 = natural or not natural amino acid free or protected side chain

or

R_3 = $(CH_2)_n$-R"

where

n = 1, 2, 3, 4, 5

R" = cyclooctyl, adamantyl, cyclohexyl, naphthyl

R" = phenyl when n is other than 1

R" = a substituted carboxyamid group when n = 1, 2

A_1 = Gln, DGln

A_2 = Trp, DTrp

A_3 = Phe, DPhe

W = CO-NR', CH_2-NR'

where

R' = H, CH_3 and their pharmaceutically acceptable salts with acids or organic or inorganic bases.

According to this invention, linear or branched C_{1-4} alkyl ar selected in the group consisting of : methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl.

Natural amino acid is selected in the group consisting of : glycine, alanine, valine, leucine, isoleucine, proline, phenylalanin , tryptophan, methionine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, in their L or D forms.

Not natural amino acid is selected in group consisting of β-alanin , D or L 2-aminoisobutyric acid, D or L 2,3-diaminopropionic acid, D or L norleucine, D or L alloisoleucine, D or L pyroglutamic acid, L or D 3-hydroxyproline, L or D 4-hydroxyproline, L or D phenylalanin substituted in the ortho, meta, or para position, L or D thienylalanine, L or D pyridylalanine, β(2- or 3-benzothienylalanine), 1,2,3,4 tetrahydroisoquinoline-3-carboxyl acid.

Among the amino acid chain protectors, the following are given special considerati n: Mbs, Mtr, NO2, Z, Tos, Pmc, For, Me, Ac, 2-

Br-Z, 2-Cl-Z, Bzl, 2,6-dichloro-Bzl, SO3H, Fmoc, OMe, OBzl, OFm, ONp, OSu.

Protected side chain of a natural or not natural amino acid means, in particular, L or D Arg (Mbs), L or D Arg(Mtr), L or D Arg(NO_2), L or D Arg (Z), L or D Arg(Tos), L or D Arg(Pmc), L or D Trp(For), L or D Trp(Mts), L or D Tyr(Me), L or D Tyr(Ac), L or D Tyr(2-Br-Z), L or D Tyr(Bzl), L or D Tyr(2,6-dichloro-Bzl), L or D Tyr(SO_3H), L or D Ser(Me), L or D Ser(Ac), L or D Ser(Bzl), L or D Ser(2,2-dichloro-Bzl), L or D Ser(SO3H), L or D Lys(Ac), L or D Lys(2-Br-Z), L or D Lys(2-Cl-Z), L or D Lys(Fmoc), L or D Lys(Z), L or D Lys(Tos), L r D Lys(Me), L or D Lys (Bzl), L or D Asp(OMe), L or D Asp(OBzl), L r D Asp(OFm), L or D Asp(ONp), L or D Asp(OSu), L or D Glu(OMe), L or D Glu(OBzl), L or D Glu(OFm), L or D Glu(ONp), L or D Glu(OSu).

Substituted carboxamide group means a $CONR_5R_6$ group, where R_5 and R_6 are equal or different and represent H or a linear or branched or cyclic alkyl, arylalkyl, aryl residue.

R_5 and R_6 together with the nitrogen atom can form a 5- or 6-terminal cycle including 4 or 5 carbon atoms or groups -$CH_2CH_2NHCH_2CH_2$-, $CH_2CH_2N(CH_3)CH_2CH_2$-, -$CH_2CH_2OCH_2CH_2$-.

In particular, NR_5R_6 can mean the residue of benzylamin , ph nyl thylamine even substituted with a hal gen, 1- r 2-naphthylamine, cycl hexylamin , cyclooctylamine, adamantanamin , adamantyl-m thylamine.

Among the c mpounds as per f rmula (I) of this inventi n, preferenc

R_1 = isobutyl

A_2 = Trp

R_3 = $(CH_2)_nC_6H_{11}$, where n = 2, 3, 4, 5; $(CH_2)_n$-(1-naphthyl), where n = 2, 3, 4, 5; $(CH_2)_n$-(1-adamantyl), where n = 1, 2, 3, 4, 5; $(CH_2)_n$-cyclooctyl, where n = 1, 2, 3, 4, 5; $(CH_2)_n$-CP_6H_5, where n = 2, 3, 4, 5; $(CH_2)_n$-CONHBzl, where n = 1, 2; $(CH_2)_n$-CONMeBzl, where n = 1, 2; $(CH_2)_n$-$CONHCH_2C_6H_{11}$, where n = 1, 2; $(CH_2)_n$-$CONMeCH_2C_6H_{11}$, where n = 1, 2; $(CH_2)_n$-CONH-CH_2(1-adamantyl), where n = 1, 2; $(CH_2)_n$-CONMe-CH_2(1-adamantyl), where n = 1,2.

In particular the following compounds are preferred:

cyclo(Leu-Cha-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp($NHCH_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp($NMeCH_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu($NHCH_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu($NMeCH_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu($NHCH_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu($NMeCH_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp($NHCH_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp($NMeCH_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Asp(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Asp(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Asp(NHCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Asp(NMeCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Glu(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Glu(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Glu(NHCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Glu(NMeCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Glu(NHCH_2(1-adamantyl))-Gln-Trp -Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Glu(NMeCH_2(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Asp(NHCH_2(1-adamantyl))-Gln-Trp -Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Asp(NMeCH_2(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Asp(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Asp(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Asp(NHCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Asp(NMeCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Glu(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Glu(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Glu(NHCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Glu(NMeCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Glu(NHCH_2(1-adamantil))-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Glu(NMeCH_2(1-adamantil))-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]Asp(NHCH_2(1-adamantil))-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NM]Asp(NMeCH_2(1-adamantil))-Gln-Trp-Phe-βAla)

cycl (Leu Ψ [CH_2NH]Asp(OBzl)-Gln-Trp-Ph -βAla)

cyclo(Leu Ψ [CH_2NMe]Asp(OBzl)-Gln-Trp-Ph -βAla)

cycl (Leu Ψ [CH_2NMe]Nal-Gln-Trp-Ph -βAla)

cyclo(Leu Ψ [CH_2NMe]Cha-Gln-Trp-Ph -βAla)

cyclo(Leu Ψ [CH_2NH]Cha-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NH]Nal-Gln-Trp-Phe-βAla)

cyclo(Leu Ψ [CH_2NMe]CH((CH_2)$_3$Bzl)CO-Gln-Trp-Phe -βAla)

cyclo(Leu Ψ [CH_2NH]CH((CH_2)$_3$Bzl)CO-Gln-Trp-Phe-βAla)

cyclo(Leu-NH-CH((CH_2)$_3$Bzl)CO-Gln-Trp-Phe-βAla)

The cyclic peptide analogues covered by the present invention can be prepared by known synthetic techniques in the solid phase or in solution. For the obtainment of linear peptides with the C-terminal carboxyl group in the form of free acid, solid supports such as resin phenylacetamidomethyl (PAM) or the resin p-hydroxymethylphenoxymethyl (Wang), can be used. In the case of PAM resin, the amine function of amino acids is protected by the t-butyloxycabonyl group which can be selectively deprotected by trifluoracetic acid, whilst final deprotection - with simultaneous peptide detachment from the polymer support - is secured by anhydrous hydrofluoric acid. In the case of the Wang resin, the amino acid amine function is protected by the 9-fluorenyl-methoxycarbonyl group (Fmoc), selectively deprotected by piperidine, whilst final deprotonation - with simultaneous peptide detachment from the polymer support - is secured by trifluoracetic acid.

In both cases, the trifunctional amino acid side chains can be protected by the known methods described by literature. For the construction of the peptide chain on the insoluble polymer support, each amin acid is made to react in th form f free cid, in the

presence of a suitable coupling agent, e.g. dicycloh xyl carbodiimide (DCC), used with additives, if any, such as hydroxybenzothiazole (HOBT) or benzothiazolyl-N-oxytridimethylaminophosphonium hexafluorophosphate (BOP); as an alternative, the amino acid can be made to react in the form f symmetric anhydride, activated ester, or according to any of th other methods described in literature. Amino acid coupling reaction completion can be ninhydrin tested, as described by E.T. Kaiser et al., Anal.Biochem., 1970, 34, 595.

Amino acids with the $R_3=(CH_2)_n-CONR_5R_6$ group as side chain can be synthesized, e.g. starting from the corresponding acid (where the α-amino and α-carboxyl groups have been pre-protected), by condensation with the suitable HNR_5R_6 amine and the use f activators such as those currently employed in peptide chemistry (BOP, PyBOP, HOBT).

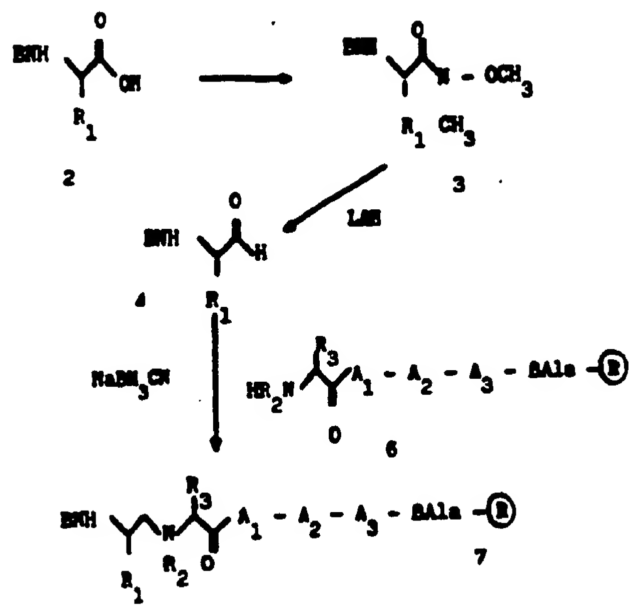
Amino acids whose side chain is represented by the $(CH_2)_n-R''$ group can be synthesized by known organic chemistry techniques, such as, e.g., those described by Evans et al., J. Am. Chem. Soc., 112 (1990) 4011-4030; G.C. Barret, Chemistry and Biochemistry of the Amino Acids, Ed. G.C. Barret, Chapman & Hall, London, 1985, 246-296.

As for th $-CH_2-NR'-$ b nd, it is synth sized according to the procedure d scribed by Sasaki and Coy, P ptides, 1987, 8, 119. Such a pr c dure was ext nd d to the synth sis in th s lid phase according to th Fmoc strategy, as described by Przewosny t al., Peptides, 1990, 370. Specifically (see scheme shown hereunder): N-

m th xymethylamide as per formula 2 is pr par d from th corresponding N-protected amino acid. The said amino acid is dissolved in methylene chloride; the solution is added with an equimolar amount of hydroxybenzotriazole and stirred for 20 minutes. Then, N-O-dimethylhydroxylamine.HCl dissolved in dichloromethane and added with an equimolar amount of a sterically hindered tertiary amine, e.g. diisopropylethylamine, is added to the said solution. The resulting mixture is kept under stirring for about 16 hours, after which it is washed with dilute aqueous HCl, with an $NaHCO3$ saturated solution, as well as with an NaCl saturated solution. The desired product can be purified, e.g. by chromatography on silica gel.

N-methoxymethylamide as per formula 3 is reduced to produce the corresponding aldehyde as per formula 4, e.g. with equimolar lithium aluminium hydride at 0°C in an ether solution. On reaction completion, the mixture is treated with a solution of acid potassium sulphate in water.

The product is then isolated by extraction, with ether, of the aqueous phase: for this purpose the ether phase is washed with dilute aqueous HCl, with $NaCO_3$ saturated solution, and with an NaCl saturated solution.



B = Boc, Fmoc R_2 = H; Me

The aldehyde as per formula 4 is allowed to react with the compound as per formula 6, or with the N-terminal end of a pentapeptide chain bound to the resin by a β-alanine residue. The initial Schiff bas is reduced in situ, e.g. by sodium cyanoborohydride, to give a modified hexapeptide bound to the resin as per formula 7. Aft r deprotection and detachment, performed as described above, th suitably freeze-dried raw peptide is purified to homogeneity, e.g. by high pressure reversed-phase preparative chromatography.

Cyclic peptid synthesis can be obtained via cyclization in solution aft r preparation - acc rding to one of th af rementioned m thods, in the solution r solid phas - of th lin ar precursor f the desired cyclic peptide. Cyclization is performed with condensing

agents and, if n cessary, by activating th C-terminal carboxyl group of the cyclic precursor.

EXAMPLE

Preparation of the cyclic peptide :

cyclo(LeuΨ [CH_2NH]Asp(NHBzl)-Gln-Trp-Phe-βAla) (ii)

a) Synthesis of the linear peptide having the following sequence:

H-LeuΨ [CH_2NH]Asp(NHBzl)-Gln-Trp-Phe-βAla-OH (i)

Synthesis of Boc-Asp(NHBzl)-OH: 323 g Boc-Asp-OBzl (Novabiochem, Switzerland) is solubilized in 70 mL dioxane; then the solution is added with 530 mg BOP, 37µL DIEA and, finally, 107 mg benzylamine. After 3 hours, the reaction mixture is dried and the residue is purified by chromatography on Merck silica gel 60 (mesh 70-230) with ethyl acetate-1/n-hexane-1 (v/v), as eluent, Rf = 0.3, 310 mg yield. Carboxyl group deprotection is obtained by dissolving 300 mg benzyl ester in 40 mL aqueous 95% ethyl alcohol and adding the solution to a suspension of 100 mg Pd/C (10% Pd) in 6 mL 95% aqueous ethyl alcohol. The environment is saturated with hydrogen and the reacting mixture is kept under hydrogen environment for 2 hours. Then, the solution is filtered and dried.

3.0 g Boc-βAla-PAM resin (Bachem, Switzerland), equal to 0.45 mmoles of amine groups, is fed to a Labortec SP 640 semi-automatic peptide synthesis reactor. The resin is washed as described in Table 1, cycles 6-7.

For resin coupling to the subsequent amino acid, symmetric anhydride is pr pared by dissolution of 0.48 g Boc-Phe-OH in 5 mL

dichloromethan . The solution temperature is brought to 0°C and added with 0.9 mL of a 1M solution of dicyclohexylcarbodiimide in dichloromethane. After 15 minutes, dicyclohexylurea is filtered and the resulting solution is added to the deprotected resin. The resin is kept under stirring at ambient temperature for 60 minutes (cycl 8). The procedure is completed by washing (cycles 9-12) and the reaction is ninhydrin-tested by the Kaiser method. In case of a negative response, the Boc group is hydrolyzed with 50% TFA (cycl s 1-4), before the subsequent amino acid coupling, which takes plac according to the described procedure. The following residues are made to react in the same order, in the quantities indicated in brackets: Boc-Trp-OH (0.548 g), Boc-Gln-OH (0.443 g), Boc-Asp(NHBzl)-OH (0.581 g). After deprotection, Boc-Leu-H (0.242 g) dissolved in a dimethylformamide solution containing 5 mL 1% acetic acid is added to the resin; 5 mL of an $NaBH_3CN$ solution (70 mg) in a dimethylformamide solution containing 5 mL 1% acetic acid is allowed to drip under stirring for 40 minutes. The resin is kept und r stirring at ambient temperature for about 6 hours. The procedure ends with washing (cycles 9-12) after which the ninhydrin test as by the Kaiser method is performed. In case of a negative response, the Boc group is hydrolyzed with 50% TFA. Then, the resin is washed (cycles 9-12) and dried und r vacuum, with th obtainment of 1.25 g dry product. For peptide d tachment from th resin, th product is placed in a Tefl n react r with 1.5 mL anisole and 0.75 mL dimethyl sulphide. The mixture temperature is brought to 50°C and 15 mL

hydrofluoric acid is distilled therein; then th mixture is kept under stirring for 60 min. in an ice bath. Hydrofluoric acid is removed by nitrogen blowing. The raw product is dried under suction for about 2 hours, is washed with ethyl ether (15 mL twice), extracted in 50% acetic acid (15 mL three times) and filtered in a fritted disc filter funnel to remove the exhaust resin. Th resulting solution is diluted with water and freeze-dried to yield 0.210 g raw product. Finally, the peptide is purified by reversed-phase liquid chromatography and characterized by analytical HPLC, Waters C18 Deltapack 3.9 x 150 mm column with an acetonitrile gradient containing 0.1% (v/v) trifluoracetic acid (phase B) vs. 0.1% (v/v) aqueous trifluoracetic acid (phase A), as well as 20 to 80% phase B, in 20 minutes, at a rate of 1 mL/min., with 210 nm UV monitoring. Retention time (Rt) = 9.2'; chromatographic purity: > 99%.

b) Cyclization of the above said peptide (i) into the cyclic peptide cyclo(Leu [CH_2NH]Asp(NHBzl)-Gln-Trp-Phe-βAla (ii)

65 mg product (i) is dissolved in 35 mL DMF. The solution is added with 47 mg PyBOP, then 32 μL DIEA. The resulting solution is kept under stirring at ambient temperature for 2 hours, then DMF is removed under vacuum and the resulting mixture freeze-dried. The cyclic peptide (ii) is purified by reversed-phase liquid chromatography and characterized by analytical HPLC, Waters C18 Deltapack 3.9 x 150 mm column with an acetonitrile gradient containing 0.1% (v/v) trifluoracetic acid (phas B) vs. 0.1% (v/v)

aqueous trifluoracetic acid (phas A), as well as 20 to 80% phas B, in 20 min., at a rate of 1 mL/min., with 210 nm UV monitoring. Retention time (Rt) = 10.6'; chromatographic purity: >99%.

By the procedure described above and using suitable reagents, the following peptides are obtained:

H-Leu Ψ [CH_2NH]Asp(NH-CH_2-(1-adamantyl))-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 9.5'; chromatographic purity: > 99%.

H-Leu Ψ [CH_2NH]Asp(NH-CH_2-C_6H_{11})-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 9.0'; chromatographic purity: > 99%.

H-Leu Ψ [CH_2NH]Glu(NHBzl)-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 9.3'; chromatographic purity: > 99%.

H-Leu Ψ [CH_2NH]Asp(NMeBzl)-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 9.8'; chromatographic purity: > 99%.

H-Leu Ψ [CH_2NH]CH((CH_2)$_3$Bzl)-CO-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 11'; chromatographic purity: > 99%.

H-Leu-Asp(NHBzl)-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 9.6'; chromatographic purity: > 99%.

H-Leu-Cha-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 9.8'; chromatographic purity: > 99%.

H-Leu Ψ [CH_2NH]Asp(OBzl)-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 10.7' ; chromatographic purity: > 99%

H-Leu Ψ [CH_2NH]Leu-Gln-Trp-DPh -βAla-OH

retenti n time (Rt) = 7.7' ; chromatographic purity: > 99%

H-Leu Ψ [CH_2NH]Lys(Z)-Gln-Trp-Ph -βAla-OH

retenti n time (Rt) = 8.9' ; chromatographic purity: > 99%

H-Leu Ψ [CH_2NH]Cha-Gln-Trp-DPhe-βAl -OH

retention time (Rt) = 9.0 ; chromatographic purity: > 99%

H-Leu Ψ [CH_2NH]Nal-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 9.9'; chromatographic purity: > 99%

H-Leu Ψ [CH_2NMe]Cha-Gln-Trp-Phe-βAla-OH

retention time (Rt) = 11.1'; chromatographic purity: > 99%

Which are cyclized into the following cyclic peptides : cyclo(Leu [CH_2NH]Asp(NH-CH_2-(1-adamantyl))-Gln-Trp-Phe-βAla)

retention time (Rt) = 11.0'; chromatographic purity: > 99%.

cyclo(Leu Ψ [CH_2NH]Asp(NH-CH_2-C_6H_{11})-Gln-Trp-Phe-βAla)

retention time (Rt) = 11.2'; chromatographic purity: > 99%.

cyclo(Leu Ψ [CH_2NH]Glu(NHBzl)-Gln-Trp-Phe-βAla)

retention time (Rt) = 10.0'; chromatographic purity: > 99%.

cyclo(Leu Ψ [CH_2NH]Asp(NMeBzl)-Gln-Trp-Phe-βAla)

retention time (Rt) = 12.5'; chromatographic purity: > 99%.

cyclo(Leu Ψ [CH_2NH]CH((CH_2)$_3$Bzl)-CO-Gln-Trp-Phe-βAla)

retention time (Rt) = 13.5'; chromatographic purity: > 99%.

cyclo(Leu-Asp(NHBzl)-Gln-Trp-Phe-βAla)

retention time (Rt) = 10.6'; chromatographic purity: > 99%.

cyclo(Leu-Cha-Gln-Trp-Phe-βAla)

retention time (Rt) = 11.2'; chromatographic purity: > 99%.

cyclo(Leu Ψ[CH_2NH]Asp(OBzl)-Gln-Trp-Phe-βAla

retention time (Rt) = 9.8' ; chromatographic purity: > 99%

cyclo(Leu Ψ[CH_2NH]Leu-Gln-Trp-DPhe-βAla)

retention time (Rt) = 9.4' ; chromatographic purity: > 99%

cyclo(Leuψ[CH_2NH]Lys(Z)-Gln-Trp-Phe-βAla)

retention time (Rt) = 10.7' ; chromatographic purity: > 99%

cyclo(Leuψ[CH_2NH]Cha-Gln-Trp-Phe-βAla)

retention time (Rt) = 11.1'; chromatographic purity: > 99 %

cyclo(Leuψ[CH_2NH]Nal-Gln-Trp-Phe-βAla)

retention time (Rt) = 13.0'; chromatographic purity: > 99%

cyclo (Leuψ[CH_2NMe]Cha-Gln-Trp-Phe-βAla)

retention time (Rt) = 12.2'; chromatographic purity: > 99%

BIOLOGICAL ACTIVITY

The ability of the peptides described in the present invention to interact with the neurokinine A receptor as agonists or antagonists was assessed through an in vitro test. The preparation used for the test was characterized by the fact that the biological response produced by tachykinins and related peptides was exclusively determined by the neurokinine A receptor (receptor NK-2). The said preparation consisted of isolated rabbit pulmonary artery affected by a dose dependent contraction brought about by tachykinins (Rovero et al., Neuropeptides, 1989, <u>13</u>, 263-270). The determination of peptide activity in the test preparation was based on the use of an NKA concentration (3 nm) causing a response equal to 45% of max. r spons . Th p ptid s consider d h r in wer add d to the preparation in growing concentrations. Th ir activity was ass ssed as inhibition of resp nse t NKA. The capacity of th peptid s described h rein to interact with th P substance receptor (receptor NK-1) as agonists or antagonists was assessed through an in vitro

test, where the biological respons produced by tachykinins and related peptides was exclusively determined at the SP receptor. The test preparation consisted of isolated guinea pig ileum affected by a dose-dependent contraction (Lee et al., Schnied. Arch. Pharmacol., 1982, 318, 281-287). The determination of peptide activity in the test preparation was based on the use of an SP methyl ester concentration (10 nm) causing a response equal to 45% of max. response (S. Dion et al., Life Sc., 1987, 41, 2269-2278). Th peptides considered herein were added to the preparation in growing concentrations. Their activity was assessed as inhibition of response to SP with satisfactory results.

The compounds covered by the invention are suitable f r therapeutical administration to higher animals and man by th parenteral, oral, dermic, nasal, inhalatory and sublingual ways, with pharmaceutical effects matching the described properties. In case of parenteral administration (intravenous, intramuscular, intradermal), sterile solutions or freeze-dried preparations of the compounds are to be used. In case of oral administration, preparations such as tablets, capsules and syrups are conveniently used. Suitably dosed ointments and creams are utilizable by the dermic way. In case of nasal instillation, inhalation, and sublingual administration, the compounds to be used are respectively aqueous solutions, aerosol preparations, or capsules.

Doses for therapeutical treatment range from 0.1 to 10 mg/kg body weight.

TABLE 1

AUTOMATIC SYNTHESIS PROCEDURE, Boc

Cycle	Reagent	Time
1	DCM	1x1 min
2	50% TFA/DCM	1x5 min
3	50% TFA/DCM	1x15 min
4	DCM	3x1 min
5	5% DIEA/DCM	2x1 min
6	DCM	2x1 min
7	DMF	2x1 min
8	Boc-AA anhydride in DCM/DMF	1x60 min
9	DMF	1x1 min
10	DCM	1x1 min
11	Repeat cycles 9 and 10	
12	DCM	3x1 min

Solvent volume: 10-20 mL/g resin

CLAIMS

1. Cyclic hexapeptides of general formula

(I) $\boxed{\text{NH-CHR}_1\text{-W-CHR}_3\text{-CO-A}_1\text{-A}_2\text{-A}_3\text{-}\beta\text{Ala}}$

where

R_1 = H, linear or branched C_{1-4} alkyl

R_3 = natural or not natural amino acid free or protected side chain

or

R_3 = $(CH_2)_n$-R"

where

n = 1, 2, 3, 4, 5

R" = cyclooctyl, adamantyl, cyclohexyl, naphthyl

R" = phenyl when n is other than 1

R" = a substituted carboxyamide group when n = 1, 2

A_1 = Gln, DGln

A_2 = Trp, DTrp

A_3 = Phe, DPhe

W = CO-NR', CH_2-NR'

wherein

R' = H, CH_3 and their pharmaceutically acceptable salts with acids or organic or inorganic bases.

2. Cyclic hexapeptides as per claim 1, wherein: linear or branched C_{1-4} alkyl are selected in the group consisting of: methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl. The natural amino acid is selected in the group consisting of:

glycine, alanin , valin , l ucin , isol ucine, proline, phenylalanine, tryptophan, methionine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, in their L or D forms.

Not natural amino acid is selected in the group consisting of:β-alanine, D or L 2-aminoisobutyric acid, D or L 2,3-diaminopropionic acid, D or L norleucine, D or L alloisoleucine, D or L pyroglutamic acid, L or D 3-hydroxyproline, L or D 4-hydroxyproline, L or D phenylalanine substituted in the ortho, meta, or para position, L or D thienylalanine, L or D pyridylalanine, β(2- or 3-benzothienylalanine), 1,2,3,4 tetrahydroisoquinoline-3-carboxyl acid.

Amino acid chain protector is selected in the group consisting of : Mbs, Mtr, NO2, Z, Tos, Pmc, For, Me, Ac, 2-Br-Z, 2-Cl-Z, Bzl, 2,6-dichloro-Bzl, SO3H, Fmoc, OMe, OBzl, OFm, ONp, OSu.

3. Hexapeptides as per claim 2, wherein the protected side chain of a natural or not natural amino acid is selected in the group consisting of : L or D Arg (Mbs), L or D Arg(Mtr), L or D Arg(NO_2), L or D Arg (Z), L or D Arg(Tos), L or D Arg(Pmc), L or D Trp(For), L or D Trp(Mts), L or D Tyr(Me), L or D Tyr(Ac), L or D Tyr(2-Br-Z), L r D Tyr(Bzl), L r D Tyr(2,6-dichl ro-Bzl), L r D Tyr(SO_3H), L r D Ser(Me), L r D Ser(Ac), L or D Ser(Bzl), L or D Ser(2,2-dichl ro-Bzl), L or D Ser(SO_3H), L r D Lys(Ac), L or D Lys(2-Br-Z), L or D Lys(2-Cl-Z), L r D Lys(Fmoc), L r D Lys(Z), L r D Lys(Tos), L r D Lys(Me), L or D Lys (Bzl), L or D Asp(OMe), L or D Asp(OBzl), L or

D Asp(OFm), L or D Asp(ONp), L r D Asp(OSu), L or D Glu(OMe), L r D Glu(OBzl), L r D Glu(OFm), L or D Glu(ONp), L or D Glu(OSu).

4. Cyclic hexapeptide as per claim 3, wherein R_1 = isobutyl.

5. Cyclic hexapeptide as per claim 4, wherein A_2 = Trp.

6. Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-C_6H_{11}, where n = 2, 3, 4, 5.

7. Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-(1-naphthyl), where n = 2, 3, 4, 5.

8. Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-(1-adamantyl), where n = 1, 2, 3, 4, 5.

9. Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-cyclooctyl, where n = 1, 2, 3, 4, 5.

10.Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-C_6H_5, where n = 2, 3, 4, 5.

11.Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-CONHBzl, where n = 1, 2.

12.Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-CONMeBzl, where n = 1, 2.

13.Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-$CONHCH_2C_6H_{11}$, where n = 1, 2.

14.Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-$CONMeCH_2C_6H_{11}$, where n = 1, 2.

15.Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-CONH-CH_2(1-adamantyl), where n = 1, 2.

16.Cyclic hexapeptide as per claim 5, wherein R_3 = $(CH_2)_n$-CONMe-

CH_2(1-adamantyl), where n = 1, 2.

17.Cyclic hexapeptide of general formula (I), as per claim 1, selected in the group consisting of :

cyclo(Leu-Cha-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp(NHCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp(NMeCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu(NHCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu(NMeCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu(NHCH_2(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu-Glu(NMeCH_2(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp(NHCH_2(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(Leu-Asp(NMeCH_2(1-adamantyl))-Gln-Trp-Phe-βAla)

18.Cyclic hexapeptide, of general formula (I), as per claim 1, selected in the group consisting of :

cyclo(LeuΨ[CH_2NH]Asp(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH_2NH]Asp(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH_2NH]Asp(NHCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH_2NH]Asp(NMeCH_2C6H11)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH_2NH]Glu(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH_2NH]Glu(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH_2NH]Glu(NHCH_2C6H11)-Gln-Trp-Ph -βAla)

cyclo(LeuΨ[CH$_2$NH]Glu(NMeCH$_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NH]Glu(NHCH$_2$(1-adamantyl))-Gln-Trp -Phe-βAla)

cyclo(LeuΨ[CH$_2$NH]Glu(NMeCH$_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NH]Asp(NHCH$_2$(1-adamantyl))-Gln-Trp -Phe-βAla)

cyclo(LeuΨ[CH$_2$NH]Asp(NMeCH$_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NH]Asp(OBzl)-Gln-Trp-Phe-βAla

cyclo(LeuΨ[CH$_2$NH]Cha-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NH]Nal-Gln-Trp-Phe-βAla)

19. Cyclic hexapeptide of general formula (I), as per claim 1, selected in the group consisting of:

cyclo(LeuΨ[CH$_2$NMe]Asp(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Asp(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Asp(NHCH$_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Asp(NMeCH$_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Glu(NHBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Glu(NMeBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Glu(NHCH$_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Glu(NMeCH$_2$C6H11)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Glu(NHCH$_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Glu(NMeCH$_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Asp(NHCH$_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]Asp(NMeCH$_2$(1-adamantyl))-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NMe]CH((CH$_2$)$_3$Bzl)CO-Gln-Trp-Phe -βAla)

cyclo(LeuΨ[CH$_2$NMe]Asp(OBzl)-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH$_2$NM]Nal-Gln-Trp-Phe-βAla)

cyclo(LeuΨ[CH_2NMe]Cha-Gln-Trp-Phe-βAla)

20.Cyclic hexapeptide, general formula (I), as per claim 1, selected among those of the group formed by

cyclo(LeuΨ[CH_2NH]CH((CH_2)$_3$Bzl)CO-Gln-Trp-Phe-βAla)

cyclo(Leu-NH-CH((CH_2)$_3$Bzl)CO-Gln-Trp-Phe-βAla)

21.Peptide preparation process as per claim 1 including peptide chain solid phase synthesis from C-terminal end to N-terminal end on an insoluble polymer support, the introduction of the iminomethylen bond, the subsequent detachment from polymer support by hydrolysis in anhydrous hydrofluoric acid and the linear peptide cyclization in polar organic solvents.

22.Peptide preparation process as per claim 1 including peptide chain solid phase synthesis from C-terminal end to N-terminal on an insoluble polymer support, the introduction of the iminomethylen bond, the subsequent detachment from polymer support by hydrolysis in trifluorac tic acid and the linear peptid cyclizati n in polar organic solvents.

23.Pharmac utical c mpositi ns f r the treatment of diseases wh re tachykinines play a pathogenic role

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/01760

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K7/64; C07K7/56; A61K37/43

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EP,A,0 412 542 (MERRELL DOW PHARMACEUTICALS INC.) 13 February 1991 * See pages 2-3 * * See page 13 (III. - VI.) * ---	1-5, 21-23
A	EP,A,0 401 507 (MERCK PATENT GESELLSCHAFT MIT BESCHRÄNKTER HAFTUNG) 12 December 1990 * See page 1 * ---	1-23
A	BRITISH JOURNAL OF PHARMACOLOGY vol. 100, 1990, pages 588 - 592 MAGGI ET AL 'Competitive antagonists discriminate between NK2 tachykinin receptor subtypes' * Page 591 (Discussion) * -----	1-23

° Special categories of cited documents : [10]

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16 NOVEMBER 1992	24. 11. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	KORSNER S.E.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9201760 SA 63764

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 16/11/92
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		CA-A-	2022740	11-02-91
		CN-A-	1049352	20-02-91
		CN-A-	1049353	20-02-91
		JP-A-	3141295	17-06-91
EP-A-0401507	12-12-90	DE-A-	3915361	15-11-90
		AU-A-	5480890	15-11-90
		CA-A-	2016355	11-11-90
		JP-A-	3002197	08-01-91

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82